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Gametophyte Morphology and Development of Six Chinese Species of *Pteris* (Pteridaceae)

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Abstract.—Spores of six Chinese species of *Pteris* (Pteridaceae) were sown on soil and subsequent gametophyte morphology and development were studied. Spores of all species are trilete, tetrahedral and with a distinct equatorial flange. Germination is *Vittaria*-type and the prothallial development is *Ceratopteris*-type in all of the species. Adult gametophytes are cordate and gametangia are of the common type for leptosporangiate ferns. Differences among species include spore size, germination time, formation time of the gametangia, gametophyte margin shape, number of archegonial neck cells and shapes of the antheridial dehiscence.

Key Words.—*Pteris*, gametophyte, *Vittaria*-type, *Ceratopteris*-type, *Pteris vittata*, *Pteris ensiformis*, *Pteris excelsa*, *Pteris fauriei*, *Pteris finotii*, *Pteris wallichiana*

The genus *Pteris* L. (Pteridaceae) is found in the tropics and is in need of redefinition (Smith *et al.*, 2006), however it is estimated to comprise about 250 species (Tryon *et al.*, 1990). Differences in characteristics of fern spore germination and gametophyte development can offer compelling criteria for taxonomic and phyletic studies (Holttum, 1949; Stokey, 1951, 1960; Atkinson and Stokey, 1964; Atkinson, 1973; Raine *et al.*, 1996; Chiou and Farrar, 1997; Chiou *et al.*, 1998; Chandra *et al.*, 2003). Type of spore germination, development of the prothallial plate and the meristematic regions, form of the mature and old thallus, type, position, and time of appearance of hairs when present, and form of the sex organs (especially the antheridium) may prove of value to the taxonomists. Pérez-García and Mendoza-Ruiz (2004) indicated that gametophytes may be useful for taxonomic and phyletic studies at the family and generic levels, as well as among species within the same genus. The combination of characters of hair type and position, margin, antheridial structure, shapes of the antheridial dehiscence, antherozoid liberation, and number of archegonial neck cells were used by Atkinson (1973), Pryer *et al.* (1995), and Pérez-García and Mendoza-Ruiz (2004) to delimit subgenera, species, or groups of species within the Thelypteridaceae.

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Though the Pteridaceae is large, studies on its gametophyte morphology are limited. However, based on the limited information, the gametophytes of *Pteris* can be summarized as the following: germination is *Vittaria*-type and the prothallial development is *Ceratopteris*-type. The adult prothallus is cordate-thalloid, with broad wings, growing very fast, with a distinct cushion. Rhizoids are nearly hyaline or pale brown, distributed in the lower surface of the cushion, with thin cell walls. The adult prothallus is naked. Gametangia are of the common leptosporangiate-type: antheridia are formed from early development stages of the prothallus. The cap cell becomes loose and is pushed off, releasing the spermatozoids. The neck of the archegonia is elongated, curving away from the apex of the prothallus (Nayar and Kaur 1971).

This study describes the gametophyte morphology and development of *P. vittata* L., *P. ensiformis* Burm, *P. excelsa* Gaud., *P. fauriei* Hieron., *P. finotii* Christ. and *P. wallichiana* Agardh.

Materials and Methods

Spores were obtained from live plants collected from several sites in China (Table 1). Fertile pinnae were kept in clean paper bags under dry conditions until spores were shed. About one week later, the sporangia and indusia were separated from the spores by a mesh with pores 0.054 mm in diameter. Spores were cultured in plastic basins (measuring 25 cm × 20 cm × 5 cm) with a sieved mixture of black soil and sand (1:1). Thickness of the mixture was about 3 cm. The surface of the mixture was made smooth and substantial and the basins were then watered. Spores of each species were sown evenly at an average density of 250–300 spores per cm^2. Basins were covered with transparent plastic film on which two to three small holes were made in order to avoid contamination and desiccation. They were placed in the dark at 25°C for 24 h then transferred to fluorescent light (10 000 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) at 25°C under a diurnal cycle of 12/12 hr. Cultures were moistened with tap water to prevent desiccation and, in the last stages, to help antheridial opening and movement of antherozoids.

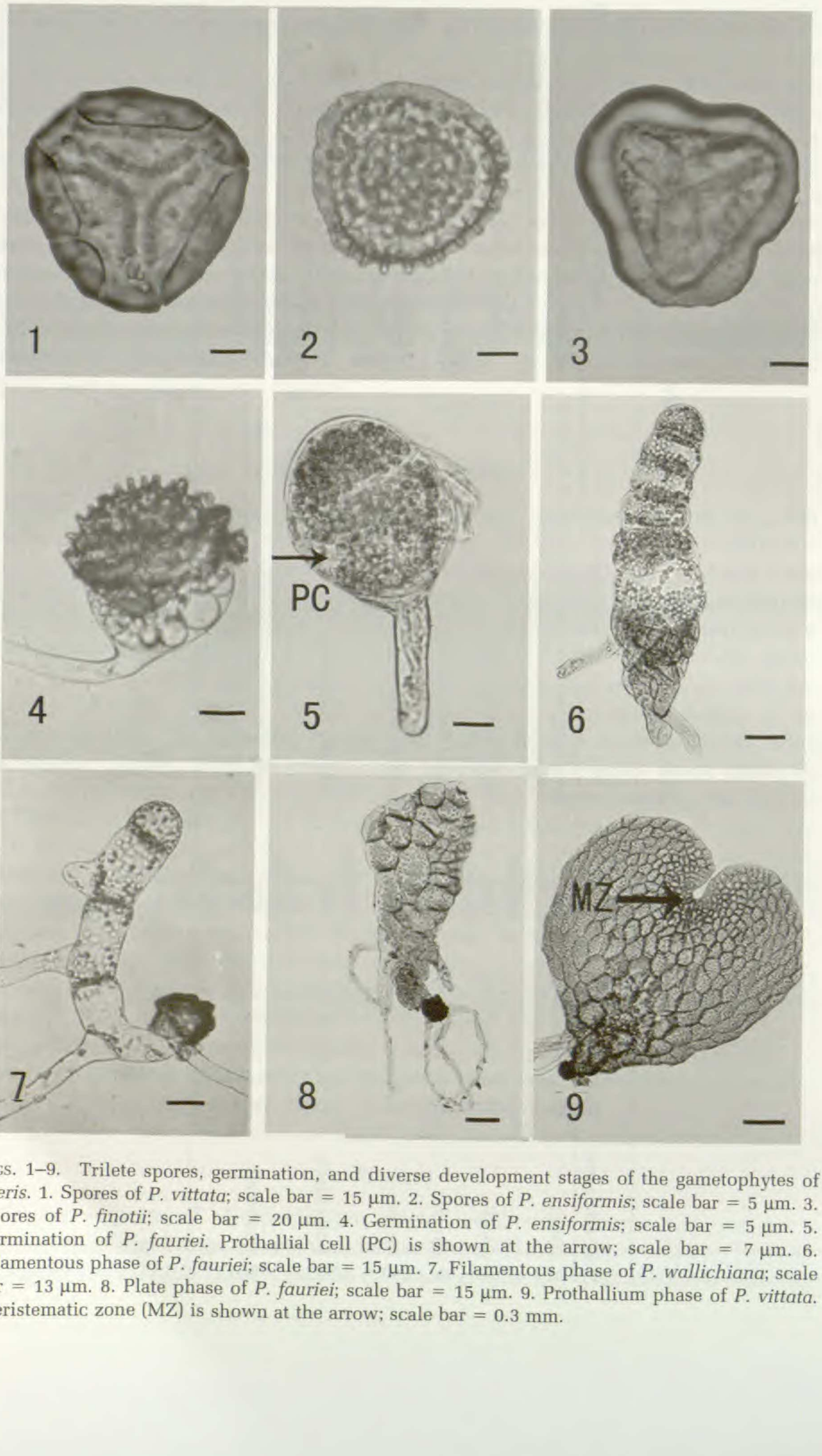
Spore sizes were measured from material in water with a compound microscope (No. XTS 20130, Beijing Tech Instrument Co., LTD) equipped with an ocular micrometer. Measurements of the spore length and width were obtained from an average sample of fifty spores per species (Table 1). Spore morphology was observed under the compound microscope from material in water. All pictures of microscopic material were taken from living materials under lab conditions with a Nikon ECLIPSE E600 camera.

Results

Spores.—Spores of all species are trilete, tetrahedral, brown and possess a distinct equatorial flange. Spores vary in size from (19) 27.4 (40) × (10) 10.3 (12) μm (*P. fauriei*) to (92) 104 (110) × (90) 96 (100) μm (*P. finotii*) (Figs. 1–3, see Table 2).

TABLE 1. Collection data for materials used in current study.

Scientific name	Collector name	Collection No. and date	Site location	Deposit herbarium	Spore numbers
P. vittata	W. L. Tim-Chun	141 04/2004	Hongkong, terraces near herbarium of Kadoorie Farm & Botanic Garden (KFBG)	Herbarium of KFBG	50
P. finotii	W. L. Tim-Chun	1894 04/2004	Fern Walk at KFBG	Herbarium of KFBG	51
P. fauriei	W. L. Tim-Chun	1941 04/2004	Orchid Fall at KFBG	Herbarium of KFBG	50
P. excelsa	B. D. Liu	151 07/2004	Yunnan, Kunming	Herbarium of Harbin Normal University	50
P. wallichiana	B. D. Liu	192 07/2004	Yunnan, Kunming	Herbarium of Harbin Normal University	49
P. ensiformis	X. C. Zhang	172 06/2004	Institute of Botany, the Chinese Academy of Sciences (IBCAS)	Herbarium of Chinese National Herbarium, IBCAS (PE)	50



FIGS. 1–9. Trilete spores, germination, and diverse development stages of the gametophytes of *Pteris*. 1. Spores of *P. vittata*; scale bar = 15 μm. 2. Spores of *P. ensiformis*; scale bar = 5 μm. 3. Spores of *P. finotii*; scale bar = 20 μm. 4. Germination of *P. ensiformis*; scale bar = 5 μm. 5. Germination of *P. fauriei*. Prothallial cell (PC) is shown at the arrow; scale bar = 7 μm. 6. Filamentous phase of *P. fauriei*; scale bar = 15 μm. 7. Filamentous phase of *P. wallichiana*; scale bar = 13 μm. 8. Plate phase of *P. fauriei*; scale bar = 15 μm. 9. Prothallium phase of *P. vittata*. Meristematic zone (MZ) is shown at the arrow; scale bar = 0.3 mm.

Germination.—Spores begin to germinate between day 2 and day 13 after they are sown (Table 2). Germination is *Vittaria*-type in all species (Figs. 4–5). Gametophytes of all the species first develop a rhizoid. Of all species, division begins in the first prothallial cell with a transverse wall and finally forms a short germ-filament, 2–25 cells long (Figs. 6–7).

Laminar phase.—The differentiation of this phase is asynchronous in all species and the development occurs between days 6 and 40 (Fig. 8; see Table 2). In *P. vittata*, as the prothallial plate grows, meristematic activity gradually becomes focused on a group of marginal cells on one side of the plate, away from the apical region. This lateral meristematic region soon locates at the bottom of a notch, which increasingly becomes more obvious as growth proceeds. The position of the meristem results in the asymmetrical young prothallus with one wing larger than the other. When the meristem is formed farther away from the apex, the thallus remains distinctly lopsided longer. The thallus becomes nearly symmetrical by growth of the sides of the wings, making the meristem nearly apical. At last, the prothallial plate becomes cordate after 5–30 days, so development of the prothallial plate is *Ceratopteris*-type (as defined by Nayar and Kaur, 1969). Then a cushion with the gametangia forms. The adult gametophyte is cordate. The time for the first adult cordiform gametophytes of all species to differentiate ranges between days 17 and 50 (Figs. 9–11, see Table 2). The prothallial development pattern of the other species is identical to *P. vittata*.

Gametangia.—Once the gametophytes have reached sexual maturity (20–90 days), the gametangia differentiation and development begins. The gametangia are all of leptosporangiate, homosporous ferns. Antheridia of all species are distributed on the lower surface of the gametophyte at the basal end of the cushion. Antheridia are globose and are composed of a basal cell, a ring cell and an opercular cell (Figs. 12–13). During antheridial dehiscence the opercular cell becomes loose and is pushed off, releasing the spermatozoids.

In all species, the archegonia differentiate at about the same time as antheridia. Archegonia are distributed on the lower surface of the gametophyte at the apical end of cushion and near the meristematic region. The necks are oriented toward the basal region of the gametophytes, with 4 rows of cells, 3–5 cells per row (Figs. 14–15).

Sporophytes.—The first sporophytes were observed by about 5–8 weeks after sowing. Fertilization occurred on almost all gametophytes to produce sporophytes.

Discussion

The spores of all species share features such as trilete spores with a distinct equatorial flange, however the spore sizes of the six species are different.

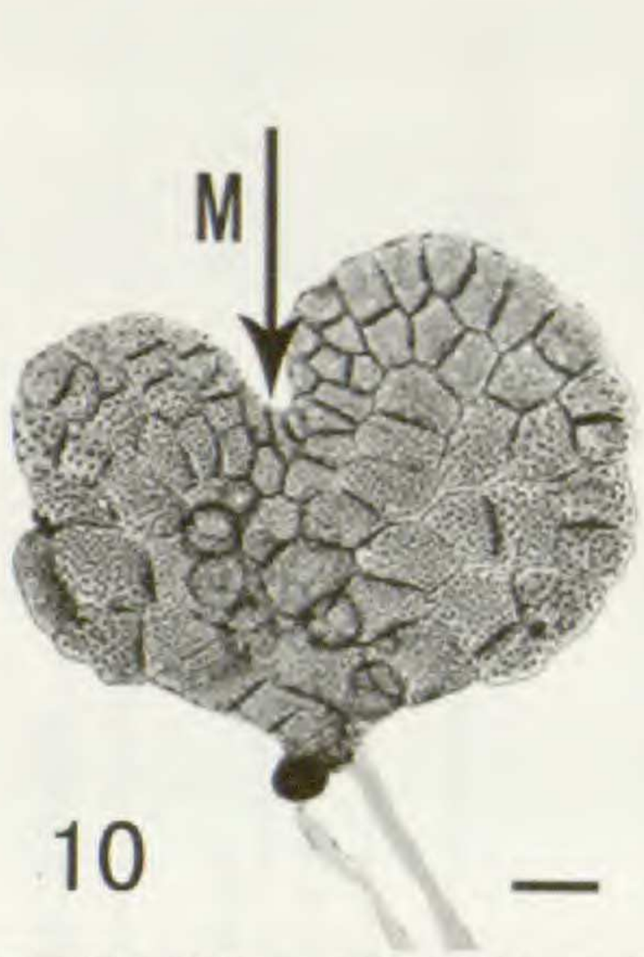
For all species, the germination pattern is of the *Vittaria*-type. It is the most common type in ferns (Nayar and Kaur, 1971). In this type, the rhizoid develops first after a wall perpendicular to the polar axis of the spores is formed. The first rhizoid of *P. fauriei* is chlorophyllous, but according to Nayar and Kaur (1971), the rhizoids of *Pteris* are nonchlorophyllous. The first

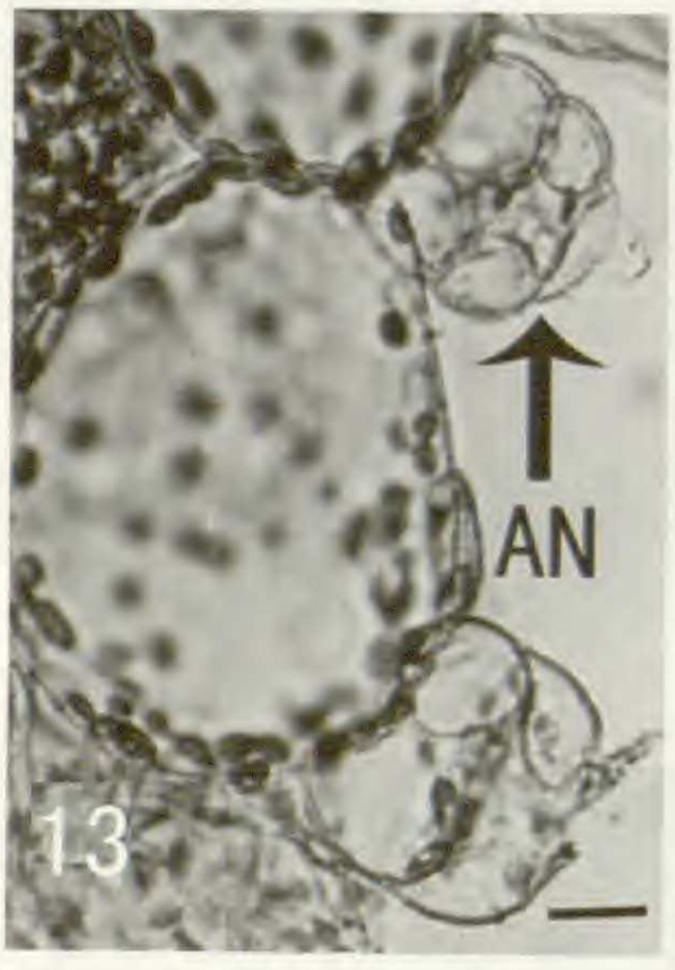
TABLE 2. Developmental stages of the gametophytes of the six species of *Pteris*.

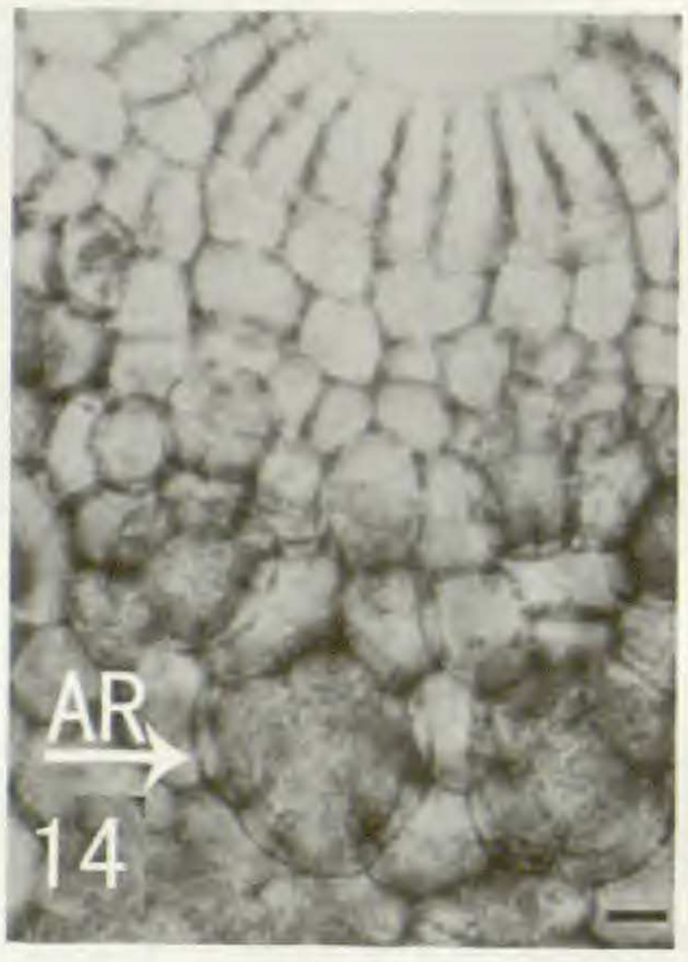
Scientific name	Spores	Germination	Filamentous phase	Plate phase	Adult phase	Gametangia
P. vittata	Trilete, tetrahedral, brown and possess a distinct equatorial flange, (90) 95 (100) × (80) 87.5 (90) μm	*Vittaria*-type, day 2–3, a rhizoid first develops and the first prothallial cell divides with a transverse wall	Germinal filaments 2–19 cells long, spore coat remains attached	*Ceratopteris*-type, about one week	17–50 days, asymmetrical spatulate to cordiform	♀♂
						20–40 days
P. finotii	Trilete, tetrahedral, brown and possess a distinct equatorial flange, (92) 104 (110) × (90) 96 (100) μm	*Vittaria*-type, day 4–6, a rhizoid first develops and the first prothallial cell divides with a transverse wall	Germinal filaments 2–12 cells long, spore coat remains attached	*Ceratopteris*-type, 10–40 days	about 26 days, asymmetrical spatulate to cordiform	♀♂
						26–60 days
P. fauriei	Trilete, tetrahedral, brown and possess a distinct equatorial flange, (19) 27.4 (40) × (10) 10.3 (12) μm	*Vittaria*-type, about one week, a rhizoid first develops and the first prothallial cell divides with a transverse wall	Germinal filaments 2–7 cells long, spore coat remains attached	*Ceratopteris*-type, 7–14 days	about 24 days, asymmetrical spatulate to cordiform	♀♂
						28–80 days
P. excelsa	Trilete, tetrahedral, brown and possess a distinct equatorial flange, (29) 31 (34) × (20) 24 (29) μm	*Vittaria*-type, day 7–11, a rhizoid first develops and the first prothallial cell divides with a transverse wall	Germinal filaments 2–22 cells long, spore coat remains attached	*Ceratopteris*-type, about 20 days	about 25 days, asymmetrical spatulate to cordiform	♀♂
						about 25 days

TABLE 2. Continued.

Scientific name	Spores	Germination	Filamentous phase	Plate phase	Adult phase	Gametangia
P. wallichiana	Trilete, tetrahedral, brown and possess a distinct equatorial flange, (44) 50 (56) × (40) 46 (51) μm	*Vittaria*-type, about day 13, a rhizoid first develops and the first prothallial cell divides with a transverse wall	Germinal filaments 2–7 cells long, spore coat remains attached	*Ceratopteris*-type, about 36 days	about 43 days, asymmetrical spatulate to cordiform	♀♂ about 36 days
P. ensiformis	Trilete, tetrahedral, brown and possess a distinct equatorial flange, (27) 30 (32) × (23) 28 (29) μm	*Vittaria*-type, about one week, a rhizoid first develops and the first prothallial cell divides with a transverse wall	Germinal filaments 2–25 cells long, spore coat remains attached	*Ceratopteris*-type, about 25 days	about 50 days, asymmetrical spatulate to cordiform	♀♂ about 90 days







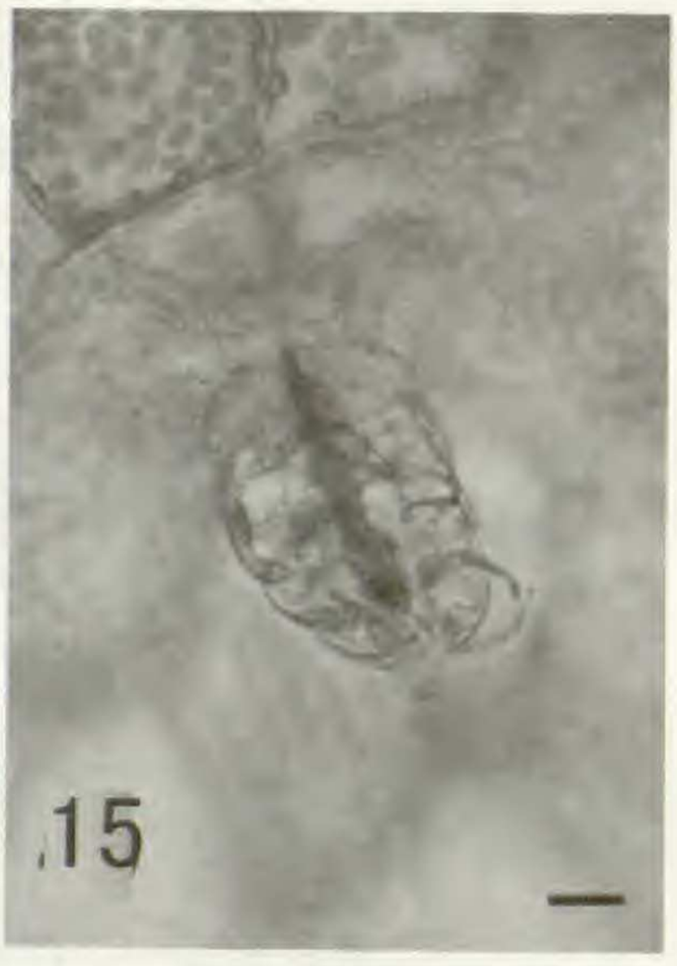


FIG. 10–15. Prothallial phases and sex organs of *Pteris*. 10. Prothallial phase of *P. fauriei*. Meristem (M) is shown at the arrow; scale bar = 0.5 mm. 11. Prothallium phase of *P. finotii*; scale bar = 0.3 mm. 12. Antheridia (AN) of *P. vittata*; scale bar = 70 µm. 13. Antheridia (AN) of *P. wallichiana*; scale bar = 13 µm. 14. Archegonia (AR) of *P. excelsa*; scale bar = 8 µm. 15. Neck of archegonia of *P. finotii*; scale bar = 20 µm.

prothallial cell divides and then the apical cell continues to divide, producing a short filament 2–25 cells long. Germination time differs among all species; spores of *P. vittata* germinate faster than the other species.

Prothallial development in all species is of the *Ceratopteris*-type in which the prothallial plate is nonmeristic at the beginning. With its growth, a multicellular meristem emerges on one side of the plate. Cell divisions in the meristem make the young thallus asymmetrical. With the growth of the smaller wing of the thallus, it becomes symmetrical. The adult gametophyte develops faster in *P. vittata* than the other species.

Sex organs are of the common leptosporangiate type. The dehiscence type in antheridia is consistent with the description given by Nayar and Kaur (1971).

The uniform development of the gametophyte in all species has been mentioned above. Distinguishing characteristics among the six species such as size of the spores, germination time, time of formation of the gametangia, thallus margin shape, number of archegonial neck cells was also observed.

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